

Chemical:

Triclopyr (Dowco 233)

2. Formulation:

98.9%

3. Citation:

Wildlife International (1980). One-Generation Reproduction Study - Mallard Duck. Project No. 103-192. January 9. Acc. No. 242368.

4. Reviewed By:

Carol Matti Natella  
Wildlife Biologist  
EEB/HED

5. Date Reviewed:

January 5, 1981

6. Test Type:

Avian Reproduction - Mallard Duck

7. Reported Results:

Examination of the number of 14 day-old survivors as a percentage of the number of eggs set for each dosage level showed a statistically significant ( $p. < .01$ ) depression at the 500 ppm dosage level, and a depression approaching statistical significance ( $p. < .02$ ) at the 200 ppm dosage level.

8. Reviewer's Conclusions:

The study is scientifically sound and shows a slight but statistically significant reproductive impairment in waterfowl at 500 ppm and a much slighter (but also statistically significant) impairment at 200 ppm. The reproductive parameters affected at 500 ppm were number of normal hatchlings and number of 14 day-old survivors. At 200 ppm, the number of 14 day-old survivors was affected. The number of eggs laid may have been affected by the test material; however, the response is not dose related. The study does fulfill the requirements for an avian reproduction study on waterfowl.

## Material/Methods

### Test Procedures

Protocol generally follows the proposed EPA guidelines of March 7, 1980. Some specifics of the study include: age of test birds at initiation of study - 6 months; duration of adult exposure prior to egg laying - 10 weeks; duration of egg collection - 10 weeks; test initiation - April 2, 1979; test termination - September 26, 1979; dosage levels - control, 100 ppm, 200 ppm and 500 ppm; birds per pen - 2 drakes, 5 hens; number of pens per treatment level - 5.

The test material was mixed with corn oil prior to mixing with aliquots of basal diet. The concentrates were frozen until utilized for weekly diet preparation. The adult ducks in both the control and experimental groups received the appropriate diets ad libitum for the duration of the study. The photoperiod for the first eight weeks of the study was 9 hours of light per day. This was increased to 17 hours per day for weeks 9 through 15 and to 22 hours for weeks 16 through 20.

Throughout incubation, the temperature was maintained at  $99.5^{\circ}\text{F} \pm 0.1^{\circ}\text{F}$  with a wet bulb humidity index of  $90.0^{\circ}\text{F} \pm 1.0^{\circ}\text{F}$ . On day 22 or 23 of incubation, the eggs were transferred to the hatcher and the temperature was lowered to  $99.0^{\circ}\text{F} \pm 1.0^{\circ}\text{F}$ , and the wet bulb humidity index was raised to  $92.0^{\circ}\text{F} \pm 1.0^{\circ}\text{F}$ .

### Statistical Analyses

The method of Cochran (Analysis of Variance for Percentages Based on Unequal Numbers, JASA, 1943, Vol. 38, pp 297-301) was used to evaluate these data. The Two-Way Analysis of Variance, the Student's t-test, and the Chi-Square test were utilized where appropriate.

Evaluation of the reproductive data and statistical analysis of the reproductive parameters: eggs laid, eggs cracked, viable embryos, live three-week embryos, normal hatchlings, hatchlings' body weight, 14 day-old survivors, 14 day-old survivors' body weight, egg weight, and eggshell thickness demonstrate that the test material caused no statistically significant reproductive impairment at the dosage levels tested. However, statistical examination of the number of 14 day-old survivors as a percentage of the number of eggs set for each dosage level showed a statistically significant ( $p < .01$ ) depression at the 500 ppm dosage level, and a depression approaching statistical significance ( $p < .02$ ) at the 200 ppm dosage level.

## Discussion/Results

Table 1A

### REPRODUCTIVE DATA - MALLARD DUCK

	Controls	DOWCO 233 (ppm)		
		100	200	500
Eggs Laid	741	595	755	567
Eggs Cracked	14	23	14	13
Eggs Set	677	522	691	501
Viable Embryos	497	423	527	377
Live Three-Week Embryos	473	404	494	345
Normal Hatchlings	287	233	259	172
14 Day-Old Survivors	277	220	240	163
Egg Laid/Hen in 10 Weeks*	30	24	30	23
14 Day-Old Survivors/Hen*	11	9	10	7

\*Based on 25 hens.

TABLE 1B

### Reproductive Success Data - Mallard Duck

	Controls	DOWCO 233 (ppm)		
		100	200	500
Eggs Laid of Theoretical Maximum (%)	42	34	43	32
Eggs Cracked of Eggs Laid (%)	2	4	2	2
Viable Embryos of Eggs Set (%)	73	81	76	75
Live Three-Week Embryos of Viable Embryos (%)	95	96	94	92
Normal Hatchlings of Live Three-Week Embryos (%)	61	58	52	50
14 Day-Old Survivors of Normal Hatchlings (%)	97	94	93	95
14 Day-Old Survivors of Eggs Set (%)	41	42	35**	33*

\*Statistically significant impairment ( $p < .01$ ).

\*\*Impairment approaching statistical significance ( $p < .02$ ).

Mature mallard ducks receiving Triclopyr at dietary concentrations of 100 ppm, 200 ppm and 500 ppm showed no symptoms of toxicity or behavioral abnormalities for the duration of the study. There was one mortality in the control group (12th week) and one mortality in the 500 ppm group (14th week). This mortality occurred during the stress of egg production, and no gross compound related abnormalities were noted upon necropsy.

There was a slight, but statistically significant ( $p. < .01$ ) reduction in feed consumption by adult birds at the 100 ppm dosage level, and a statistically significant ( $p. < .01$ ) difference in the body weight of adult birds at the 500 ppm dosage level.

#### Reviewer's Evaluation

##### A. Test Procedure

Generally, the test procedure complies with the recommended EPA 1980 protocol. Egg fertility of the controls (as measured by percent of viable embryos) at 73% was slightly below the normal fertility value for mallards - 85 to 98%.

##### B. Statistical Analysis

A Chi-square test was performed on the reproductive data (Table 1A). Significant differences were found between 500 ppm and the control for number of normal hatchlings and number of 14 day-old survivors. Significant differences were noted between 200 ppm and the control for number of 14 day-old survivors. Number of eggs laid in the 100 and in the 500 ppm group (but not in the 200 ppm group) also differed significantly from the control.

Statistical analyses were performed on the number of 14 day-old survivors as a percentage of eggs set for the control and for the 500 ppm dose level. A  $t$  test was performed by converting the percentage values (% 14 day survivors/eggs set) for each week lot within the 500 ppm treatment group and the control group to arc sine. The  $t$  test was performed on these figures and a significant difference was found at the 5% level.

The EEB was not able to duplicate the exact procedure used by the author of this study. However, using a Chi-square test and a  $t$  test, the EEB's conclusions are much the same as those of the author - that a slight reproductive impairment exists at 500 ppm and a slighter impairment exists at 200 ppm.

##### C. Conclusions

1. Category: Core
2. Rationale: N/A
3. Repairability: N/A

1 D.F. = 3.94  
3 D.F. = 7.81

*mallard*

.000	C O N	741.000	14.000	677.000	497.000	473.000	287.000	277
.000	1 0 0	595.000	23.000	522.000	423.000	404.000	233.000	220
.000	2 0 0	755.000	14.000	691.000	527.000	494.000	259.000	240
.000	5 0 0	567.000	13.000	501.000	377.000	345.000	172.000	163

0.000	TOTALS	2658.000	64.000	2391.000	1824.000	1716.000	951.000	90
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4.746	CHISQ3	42.707	7.245	8.534	4.198	6.738	13.798	1
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000	C O N	741.000	14.000	677.000	497.000	473.000	287.000	277.
.000	1 0 0	595.000	23.000	522.000	423.000	404.000	233.000	220

023	CHISQ	15.955	4.786	4.730	2.488	2.420	0.025	0.
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000	C O N	741.000	14.000	677.000	497.000	473.000	287.000	277.
.000	2 0 0	755.000	14.000	691.000	527.000	494.000	259.000	240

174	CHISQ	0.131	0.002	0.012	1.290	0.418	3.162	5.
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000	C O N	741.000	14.000	677.000	497.000	473.000	287.000	277.
.000	5 0 0	567.000	13.000	501.000	377.000	345.000	172.000	163

727	CHISQ	23.147	0.259	3.237	0.049	1.223	9.942	10.
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END OF EXECUTION  
CPU TIME: 2.40 ELAPSED TIME: 5:22.14  
EXIT  
@TERMINAL (MODE IS) WIDTH (OF LINE IS) 100  
@

*Normal 14 day-old  
Hatchlings*

1 D

3 D

EXECUTE AVES.FOR  
LINK: Loading  
[LNKXCT AVES execution]

WILDLIFE INTERNATIONAL  
IN COOPERATION  
WITH THE ECOLOGICAL EFFECTS BRANCH

5

USERID : EPABT1  
ACCOUNT ?  
PASSWORD?  
SPECIFY GLOBAL FORMAT FOR SAVE COMMANDS  
REPLY - DEFAULT, EDIT, TSO, CARD, OR PRINT  
FORMAT? card  
RECOVERY COMPLETED  
AUTO PROFILE NOT EXECUTED DUE TO RECOVERY  
COMMAND ? clear text  
COMMAND ? \*\*\*

1. ? x 31.31  
2. ? x 42.7  
3. ? x 40.98  
4. ? x 44.43  
5. ? x 42.71  
6. ? x 39.82  
7. ? x 40.4  
8. ? x 36.9  
9. ? x 37.5  
10. ? x 37.5  
11. ? y 21.97  
12. ? y 32.58  
13. ? y 40.98  
14. ? y 32.58  
15. ? y 42.71  
16. ? y 38.06  
17. ? y 35.06  
18. ? y 39.23  
19. ? y 33.2  
20. ? y 30.66  
21. ? \*\*\*

COMMAND ? save dataset scratch card  
VOLUME? 55

55: ILLEGAL VOLUME

COMMAND ? save dataset on user55 card scratch

"DATASET" REPLACED ON USER55

COMMAND ? use ttest clear

COMMAND ? run notify

539 IS YOUR JOB NUMBER.

COMMAND ? \*\*\*

JOB 539 EPABTRXX ENDED AT DEFAULT

COMMAND ? fetch 539 clear

COMMAND ? 1 189/1 unn

1 STATISTICAL ANALYSIS SYSTEM 1  
9:50 THURSDAY, OCTOBER 16, 1980

# TTEST PROCEDURE

VARIABLE: RESPONSE

VARIABLE	N	MEAN	STD DEV	STD ERROR
X	10	39.42500000	3.79666008	1.20060934
Y	10	34.70300000	6.01065545	1.90073614

VARIANCES	T	DF	PROB > T
UNEQUAL	2.1004	15.2	0.0528
EQUAL	2.1004	18.0	0.0501

FOR H0: VARIANCES ARE EQUAL, F°= 2.51 WITH 9 AND 9 DF PROB > F°= 0.1873  
COMMAND ?

Hallard Duck

